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Please replace the paragraph on page 69, beginning with "Example 30", with the
following replacement paragraph:

## EXAMPLE 30

Selection of Mutagenized scFv-KJ16/Yeast by Fluorescence-Activated Cell Sorting

An *E. coli* mutator strain has been used to mutagenize an scFv for affinity maturation by
phage display (Low et al., 1996). This approach was successful in identifying a mutant
of scFv-4-4-20 with higher affinity for fluorescein using yeast display. A strength of this
mutagenesis approach is its simplicity, requiring only *E. coli* transformation and cell
growth. Furthermore, the *E. coli* mutator strain introduces mutations throughout the
expression plasmid, and therefore does not bias changes to portions of the scFv
believed to be important for determining binding characteristics. Whether this aspect of
mutator strain mutagenesis is advantageous depends on the ability to identify key
residues that might influence antigen binding, based on available structural information.
Examination of published affinity maturation studies suggest that the location of such
residues, generally in non-contact residues, is not yet predictable *a priori* (Hawkins et
al., 1993, Patten et al., 1996, Schier et al., 1996, Thompson et al., 1996, Yang et al.,
1995, Yelton et al., 1995).

Please replace the first paragraph on page 71 with the following replacement
paragraph:

To apply this strategy to scFv-KJ16, the scFv-KJ16/Aga2 plasmid was propagated in
the *E. coli* mutator strain XL1-Red (Strategene) for six cycles of growth. This procedure
was predicted to introduce an average of two to three point mutations in the scFv coding
sequence, based on a mutation rate per cycle of 1 in 2000 bps. The resultant plasmid
preparation was transformed into yeast yielding a library size of approximately $3 \times 10^5$
transformants. In other work, larger libraries ($10^7$) have been obtained by further
optimization of transformation procedures and by pooling independent transformations.
This number does not represent an upper size limit for library construction, as further
efforts at optimization and scaleup could be straightforwardly applied.

Please replace the paragraph on page 71, line 13 through page 72, line 9 with the
following replacement paragraph:

The mutagenized yeast library was subjected to four successive cycles of sorting and
amplification, using a double stain for anti-c-*myc* antibody binding (FITC) and
biotinylated-scTCR binding (PE). Biotinylated TCR was used at a 1:5000 dilution (~10
nM) that yielded just below the detectable threshold of binding by wt scFv-KJ16/yeast
(Figure 13). The two channel fluorescence profiles of the mutated scFv-KJ16 sample
after one sorting cycle (Figure 14A) and after four sorting cycles (Figure 14B) are
shown. Cells that exhibited fluorescence above the diagonal window shown in Figure